GATA factors participate in tissue-specific immune responses in *Drosophila* larvae

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Drosophila **responds to infection by producing a broad range of antimicrobial agents in the fat body and more restricted responses in tissues such as the gut, trachea, and malpighian tubules. The regulation of antimicrobial genes in larval fat depends on linked Rel/NF-κB and GATA binding sites. Serpent functions as the major GATA transcription factor in the larval fat body. However, the transcriptional regulation of other tissue-specific responses is less well understood. Here, we present evidence that dGATAe regulates antimicrobial gene expression in the midgut. Regulatory regions for antimicrobial genes *Diptericin* and *Metchnikowin* require GATA sites for activation in the midgut, where Grain (dGATAc), dGATAd, and dGATAe are expressed in overlapping domains. Ectopic expression of dGATAe in the larval fat body, where it is normally absent, causes dramatic up-regulation of numerous innate immunity and gut genes, as judged by microarray analysis and *in situ* hybridization. Ectopic dGATAe also causes a host of symptoms reminiscent of hyperactive Toll (Toll^{10b}) mutants, but without apparent activation of Toll signaling. Based on this evidence we propose that dGATAe mediates a Toll-independent immune response in the midgut, providing a window into the first and perhaps most ancient line of animal defense.**

dGATAe | innate immunity | midgut | Toll pathway | melanin

Insects encounter infectious agents in the form of ingested microbes, inhaled fungal spores, bites from predators such as mites, and parasites. *Drosophila* counteracts with a sophisticated immune response that includes blood clotting cascades, melanin production, antimicrobial peptide (AMP) synthesis, and a parasite encapsulation response mediated by lamellocytes (1, 2). The fat body plays a vital role in the systemic immune response by producing multiple classes of AMP genes with distinct target specificities. More recently, the barrier epithelia such as the trachea, gut, and cuticle have been found to activate subsets of AMP genes in response to localized infections (3–5). For example, microbial infection provokes the induction of a *Drosomycin-GFP* reporter in the larval trachea (3, 5). Ingestion of the Gram-negative bacterium *Erwinia carotovora* causes induction of *Diptericin*- and *Attacin-GFP* reporters in the larval midgut (5).

Antimicrobial gene activation in the fat body depends on the nuclear transport of the Rel transcription factors Dif, Dorsal, and Relish (6–8). Fungi or Gram-positive bacteria induce Toll signaling, which triggers Dif and Dorsal transport. Infection by Gram-negative bacteria leads to the processing and transport of Relish via the Imd pathway. Rel factors are not sufficient for induction of the immune response. The 5′ regulatory regions of many AMP genes contain closely linked Rel and GATA binding sites. Serpent (*srp*) is the principal GATA factor expressed in the fat body, and there is evidence that Rel–Serpent synergy is essential for a robust immune response (9). This synergy appears to depend on specific arrangements of linked Rel and GATA binding sites in the regulatory regions of select immunity genes (10). The activation of AMP genes in tissues other than the fat body raises the question of whether GATA factors are also required in localized immune responses. Perhaps different tissue-specific GATA factors are responsible for activating subsets of immunity genes in the barrier epithelia.

Members of the GATA transcription factor family contain one or two zinc fingers with the amino acid sequence $CysX_2CysX_{17}CysX_2Cys$ and can bind the DNA sequence (A/T) GATA (A/G) (11, 12). These factors play crucial roles in cell differentiation and proliferation and are conserved among fungi, plants, insects, and mammals (13). *D. melanogaster* has five GATA factors: Pannier (dGATAa), Serpent (dGATAb), Grain (dGATAc), and dGATAd and dGATAe, which were discovered by using computer-based searches (14). Recent evidence suggests that Serpent activates dGATAe in the early endoderm, where it is required for activation of midgut genes (14, 15).

We investigated the possibility that immune responses in barrier epithelia, specifically in the midgut, require different tissue-specific GATA factors. Evidence is presented that dGATAe mediates an immune response in the gut. Mutations in GATA binding sites abolish the gut response of *lacZ* fusion genes containing either *Diptericin* or *Metchnikowin* 5′ regulatory sequences. dGATAe exhibits restricted expression in the proventriculus, midgut, and malpighian tubules. Ectopic expression of dGATAe in the fat body causes a severe phenotype including melanized tissues, lamellocyte differentiation, disorganization of the fat body, and constitutive expression of the *Drosomycin* (*Drs*) gene. A similar phenotype results from constitutive activation of Toll signaling (Toll^{10b} mutant). However, RT-PCR assays suggest that the dGATAe and Toll^{10b} phenotypes arise from differing molecular causes. In particular, Toll^{10b} mutants display up-regulation of many Toll signaling components, including the genes for Spatzle, Toll, Cactus, Relish, Dorsal, and Dif. In contrast, ectopic dGATAe does not alter the expression of any of these genes. Instead, ectopic dGATAe causes activation of numerous innate immunity and midgut genes according to microarray analysis. We propose that dGATAe activates midgut-specific antimicrobial genes independent of the known Imd and Toll signaling pathways.

Results

Regulation of Immunity Genes in the Midgut Depends on GATA Sites. The minimal *Diptericin* (*Dpt*) and *Metchnikowin* (*Mtk*) 5′ regulatory regions activate a *lacZ* reporter gene in the midgut regions of larvae that ingest the bacterial entomopathogen *E. carotovora* (Fig. 1). The *Dpt* regulatory region contains two Rel binding sites and a single GATA site (Fig. 1*A*). The GATA site is arranged in the same orientation as the closest Rel site. Point mutations that abolish the core GATA recognition sequence eliminate induction of *lacZ* expression in the midgut (Fig. 1*B*).

Similar results were obtained with the *Mtk* regulatory region (Fig. 1 *C* and *D*). The minimal enhancer contains three Rel sites

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Abbreviation: UAS, upstream activating sequences.

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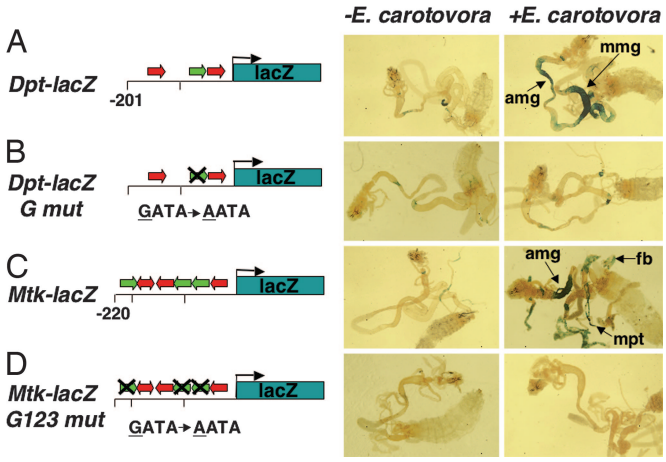

Fig. 1. GATA binding sites are essential for immune responses in the gut. (*A*) The 201-bp region from the 5′ flanking region of *Dpt* contains two Rel sites (red arrows) and one GATA site (green arrow). This region drives strong *lacZ* expression in the anterior and middle midgut (amg and mmg, respectively) of third-instar larvae fed *E. carotovora*. Weak staining occurs in the midgut of control larvae not fed bacteria. (*B*) Point mutation in the GATA site of the *Dpt* regulatory region causes loss of *lacZ* expression in the midguts of infected larvae. (*C*) The 220-bp region 5′ of *Mtk* contains three Rel and GATA sites and drives *lacZ* expression in the anterior midgut upon ingestion of *E. carotovora*. This midgut staining is generally weaker than that seen for *Dpt-lacZ*. The fat body (fb) also shows *lacZ* expression upon infection. Staining is detected in the posterior proventriculus and weakly detected in the malpighian tubules of control larvae not fed bacteria. (*D*) Mutation of all three GATA sites in the *Mtk* regulatory region abolishes *lacZ* expression in every tissue.

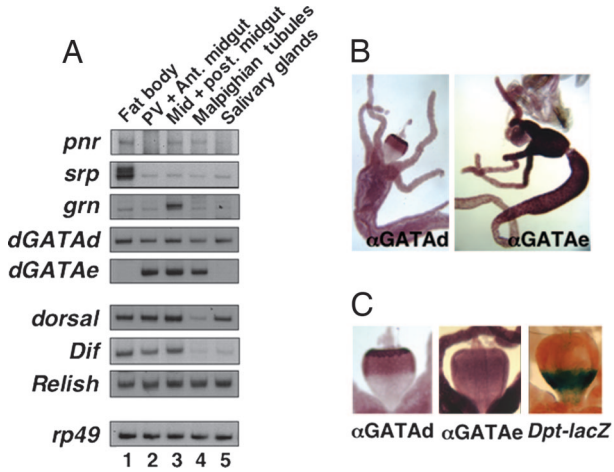

Fig. 2. Tissue-specific localization of *Drosophila* GATA family members. (*A*) Semiquantitative radioactive RT-PCRs performed with total RNA extracted from the indicated tissues. PV refers to proventriculus. A ribosomal protein gene (*rp49*) was used as a loading control. *srp* transcripts are most abundant in the fat body (lane 1). Transcripts for *grn* appear to be midgut-specific (lane 3), whereas *dGATAe* is detectable throughout the midgut (lanes 2 and 3) and the malpighian tubules (lane 4). *dGATAd* is detectable in every tissue tested (lanes 1–5). The three Rel factors *dorsal*, *Dif*, and *Relish*, are detectable in all tissues at varying levels. (*B*) *In situ* hybridizations of larval midguts with DIG-labeled *dGATAd* and *dGATAe* antisense probes. (*Left*) *dGATAd* expressed in the anterior midgut, fading along the gastric caecae. Two sharp domains with different staining intensities appear in the anterior proventriculus. (*Right*) *dGATAe* present at high levels in the anterior midgut, with reduced levels posterior to this region, along the gastric caecae, and in the proventriculus. (*C*) Close-up view of *dGATAd* and *dGATAe* RNA localization in the proventriculus. The domain of *dGATAd* expression (*Left*) apparently complements several enhancer-*lacZ* staining patterns, including *Dpt-lacZ* (*Right*). *dGATAe* is expressed in a graded fashion throughout this organ (*Center*).

linked to three GATA sites. Ingestion of *E. carotovora* causes induction of *lacZ* in the anterior midgut and fat body (Fig. 1*C*). Point mutations in all three GATA sites abolish this induction (Fig. 1*D*). It is likely that Serpent is the GATA factor responsible for induction in the fat body because it is strongly expressed in the tissue.

Tissue-Specific Expression of *Drosophila* GATA Factors. To determine whether Serpent might regulate *Dpt-lacZ* and *Mtk-lacZ* induction in the gut, RT-PCR assays were done to identify sites of the expression of all five GATA family members in the *Drosophila* genome (Fig. 2*A*). Expression of *srp* is primarily restricted to the fat body (lane 1), and there is little expression in the anterior or more posterior portions of the midgut (lanes 2 and 3). Thus, it is unlikely that *srp* mediates immunity gene expression in the gut.

Instead, there is strong expression of two other GATA factors in regions of the gut that show an immune response, dGATAe and Grain. *dGATAe* is expressed in three regions: the proventriculus plus anterior midgut (lane 2), middle and posterior midgut (lane 3), and malpighian tubules (lane 4), whereas *grn* is expressed solely in the middle plus posterior midgut (lane 3). It is therefore possible that dGATAe and Grain work together to induce immunity gene expression in posterior portions of the midgut, whereas dGATAe functions alone in the anterior midgut, proventriculus, and malpighian tubules. *dGATAd* is constitutively expressed in all tissues surveyed.

To visualize the spatial localization of *dGATAd* and *dGATAe* transcripts, *in situ* hybridizations with DIG-labeled antisense RNA probes were performed on dissected larval midguts (Fig. 2 *B* and *C*). *dGATAd* is expressed in two bands with different intensities around the anterior proventriculus (Fig. 2*B Left*). Additional staining appears in the anterior midgut. *dGATAe* is strongly expressed in the anterior midgut, with weaker expression throughout the proventriculus and gastric caecae (Fig. 2*B Right*). It is possible that dGATAd functions as a repressor to restrict gene expression to discrete regions of the gut. For example, ingestion of *E. carotovora* induces *Dpt-lacZ* within posterior regions of the proventriculus (Fig. 2*C Right*), even though *dGATAe* is expressed throughout this organ in both anterior and posterior regions (Fig. 2*C Center*). This expression appears to complement sites of *dGATAd* mRNA accumulation (Fig. 2*C Left*).

Ectopic Expression of dGATAe Leads to a Toll^{10b} Phenotype. The preceding analysis raises the possibility that different GATA transcription factors mediate immune responses in different tissues. dGATAe was selected for further study because it is expressed in regions of the gut reacting to infection. The entire dGATAe protein coding sequence was placed under the control of yeast upstream activating sequences (UAS) and misexpressed by using different *Gal4* drivers (Fig. 3).

Constitutive dGATAe expression was achieved by using a *Gal4* driver under the control of heat shock *hsp70* 5′ regulatory sequences. When *hsp70-Gal4*;*UAS-dGATAe* strains are raised at room temperature, all progeny die during the first larval instar, indicating leaky expression from the *hsp70* regulatory sequences. When raised at 18°C, the larvae develop normally. Heat shock was applied for 30 min at 30°C, and larvae were subsequently transferred to room temperature for 18 h. Third-instar larvae treated in this fashion displayed a number of abnormalities (Fig. 3 *A* and *B*), including melanization of the trachea and hindgut. All of the larvae died prior to pupation.

To determine the site of the ectopic *dGATAe* expression causing the mutant phenotype, a *Gal4* driver was used that causes selective misexpression in the developing fat body, salivary glands, and blood. The *P{GawB}c754* driver induces such

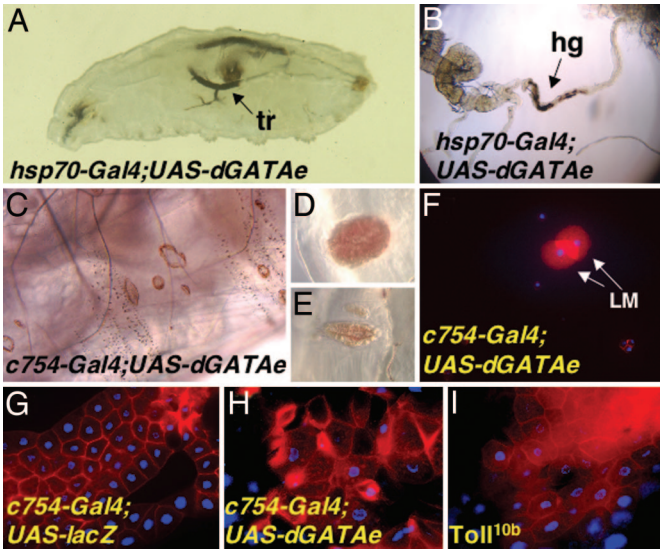

Fig. 3. dGATAe misexpression causes severe immunological defects. (*A* and *B*) Flies carrying an *hsp70-GAL4* driver were mated with flies carrying a *UAS-dGATAe* cassette. The trachea (tr, *A*) and hindgut (hg, *B*) of the third-instar progeny become melanized 18 h after heat shock. Trachea melanization first appears in the posterior spiracles of most larvae, although in this example it has begun in the middle. All larvae die prior to pupation. (*C–I*) The *P{GawB}c754* strain produces Gal4 in the fat body, blood, and salivary glands of second- and third-instar larvae. This strain was mated with *UAS-dGATAe* transgenic lines. Many of the offspring (20–50%) develop melanized spots on the cuticle (*C–E*) whereas a few (≈10%) exhibit small melanized regions within the hindgut. The cuticle lesions are typically oriented along the anterior–posterior axis, possibly in response to the crawling action of the larvae. (*F*) Blood smears stained with rhodamine-phalloidin (red) and DAPI (blue) reveal the presence of lamellocytes (LM), typically a response to parasite infection. (*G–I*) The fat body, normally a uniform monolayer of cells (*G*), becomes disorganized on dGATAe expression (*H*). Rhodamine–phalloidin (red) and DAPI (blue) staining of the tissue reveals enlarged cells and nuclei, similar to Toll^{10b} fat (*I*).

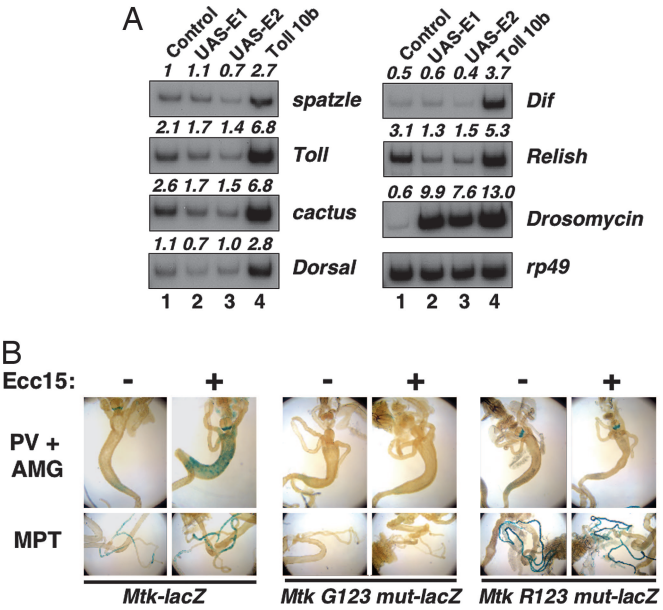

Fig. 4. dGATAe causes a Toll^{10b} phenotype without up-regulation of pathway components. (*A*) Total RNA was purified from third-instar larval fat and used in RT-PCR assays with primers specific to the indicated genes. Lane 1, yw control; lanes 2 and 3, offspring of two independent *UAS-GATAe* lines mated with *P{GawB}c754* flies; lane 4, Toll^{10b}. Results were quantified, normalized to *rp49*, and the relative intensities reported above each band. (*B*) Larvae bearing the *Mtk-lacZ* transgene were fed *Erwinia* and compared with larvae bearing mutant versions of the *Mtk* regulatory region. The leftmost panels depict *Mtk-lacZ*. Constitutive *lacZ* expression is seen in the proventriculus (PV) and malpighian tubules (MPT) with inducible expression in the anterior midgut (AMG). The center panels show the *Mtk* regulatory DNA bearing three mutated GATA sites. All *lacZ* expression is abolished. The rightmost panels show the *Mtk* regulatory DNA bearing three mutated Rel sites. Constitutive proventriculus expression and enhanced malpighian tubules expression occur, but inducible anterior midgut expression disappears.

expression as early as the second instar stage (16). The resulting climbing larvae display a consistent syndrome of defects. The cuticle is extensively spotted and scarred (Fig. 3 *C–E*), and there are melanized portions of the hindgut and excessive lamellocytes (Fig. 3*F*, LM). These observations suggest that the ectopic expression of dGATAe causes a systemic immune response in the absence of infection.

The fat body expressing dGATAe displays a number of abnormalities. The normal fat body is a single-layered tissue (Fig. 3*G*), but in response to dGATAe expression it exhibits buckling and regions composed of multiple cell layers (Fig. 3*H*). In addition, DAPI staining reveals nuclei that are larger than normal, suggesting an additional round of endoreplication. The general appearance of the fat body is similar to that seen in Toll^{10b} mutants (Fig. 3*I*). The abnormal fat body morphology, combined with tissue melanization and lamellocytes, raises the possibility that ectopic dGATAe expression induces constitutive Toll signaling, as seen in Toll^{10b} mutants.

Ectopic dGATAe Does Not Induce Toll Signaling. RT-PCR assays were performed with RNA extracted from fat bodies of *P{GawB}c754;UAS-dGATAe* and Toll^{10b} larvae (Fig. 4). As documented previously (17), constitutive Toll signaling augments the steady-state mRNA levels of different components of the Toll pathway, including the Spatzle ligand, the Toll receptor itself, the Cactus inhibitor, and all three Rel-containing transcription factors, Dorsal, Dif, and Relish (Fig. 4; compare lane 4 with lane 1). None of these genes is up-regulated in either one of two lines containing the *UAS-dGATAe* fusion gene inserted in different regions of the genome and driven by *P{GawB}c754* (Fig. 4; compare lanes 2 and 3 with lane 1). This is not because dGATAe is only transiently expressed compared with Toll^{10b}, because *P{GawB}c754;UAS-Toll^{10b}* larvae also show heightened levels of Toll signaling components (data not shown). The only obvious link between the two genotypes is the constitutive expression of *Drs* in the absence of infection. These results suggest that the phenotypes are only superficially similar and arise from distinct underlying causes.

To further investigate the possibility that dGATAe acts independent of Toll signaling, we tested the midgut response of the *Mtk 5′* regulatory sequence bearing mutations in the GATA and Rel binding sites (Fig. 4*B*). The intact *Mtk* regulatory region drives constitutive *lacZ* expression in the proventriculus and malpighian tubules. Upon infection, additional *lacZ* expression occurs in the anterior midgut (Fig. 4*B*, leftmost panels). Mutation of the three consensus GATA sites within this sequence abolishes *lacZ* expression in every tissue (Fig. 4*B*, center panels). Mutation of the three consensus Rel sites, however, only disrupts inducible anterior midgut expression (Fig. 4*B*, rightmost panels). The expression in the proventriculus remains, and the malpighian tubule expression becomes stronger, suggesting Rel factor-dependent and -independent domains of gene activity.

Ectopic dGATAe Induces Innate Immunity and Midgut Genes. Microarray assays were used to find downstream targets of ectopic dGATAe. RNA was extracted from uninfected fat bodies of *P{GawB}c754;UAS-dGATAe* climbing larvae, labeled, and hy-

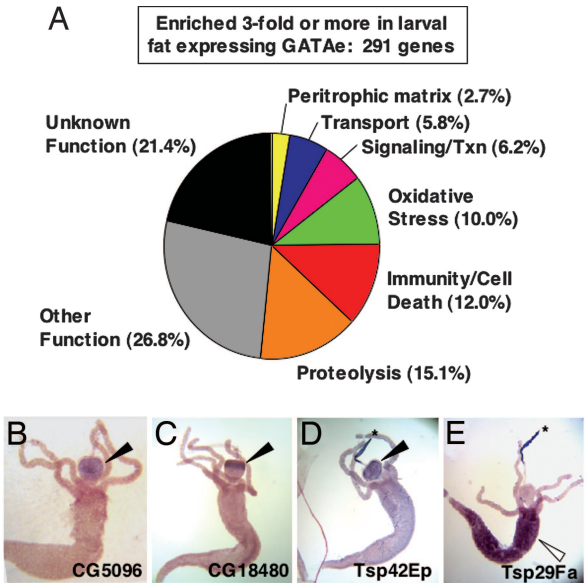

Fig. 5. Microarray analysis of ectopic dGATAe in the fat body. Total RNA was extracted from third-instar larval fat misexpressing dGATAe (*P{GawB}c754;UAS-dGATAe*) and compared with normal *yw* fat. To control for line effects and dissection inconsistencies, only the genes enriched 3-fold or greater and shared by two independent *UAS-dGATAe* lines are reported. The complete list of genes and fold induction in both arrays can be seen in Table 1. (*A*) Genes responding to ectopic dGATAe are categorized based on known function or conserved domains highlighted by BLAST searches. (*B–F*) An *in situ* hybridization of array hits reveals midgut-specific expression patterns. *CG5096*, *CG18480*, and *Tsp42Ep* are expressed in the larval proventriculus (*B–D*, filled arrowheads), whereas *Tsp29Fa* is expressed in the anterior midgut (*E*, open arrowhead). Both tetraspanins are also expressed in the esophagus (*D* and *E*, asterisk).

bridized to Affymetrix (Santa Clara, CA) microarrays containing all of the predicted protein coding genes in the *Drosophila* genome. The data were compared with normal *yw* fat body RNA by using robust multiarray analysis (18). Nearly 300 genes displayed 3-fold or greater up-regulation on dGATAe misexpression (Fig. 5*A* and Table 1, which is published as supporting information on the PNAS web site) and are grouped into categories based on known or predicted functions.

A number of the genes induced by dGATAe misexpression may play a role in digestion, including numerous proteases (44 genes) and several lipases (9 genes). Genes encoding components of the peritrophic matrix, a protective sheath secreted by the proventriculus, are also highly induced (as much as 231-fold). *In situ* hybridizations confirm midgut-specific expression patterns for many of these genes. A survey of the BDGP *in situ* database (www.fruitfly.org/cgi-bin/ex/insitu.pl) turns up patterns for 48 hits from the array, 34 of which (71%) show late embryonic midgut expression (marked as double asterisks in Table 1). Hence, dGATAe is able to activate a large number of gut genes in the fat body. We independently confirmed gut-specific expression for additional array targets by means of *in situ* hybridization of larval midguts. CG5096, CG18480, and Tsp42Ep are transcribed in the larval proventriculus (Fig. 5 *B–D*), and Tsp29Fa is expressed in the anterior midgut of larvae (Fig. 5*E*).

Genes known or predicted to have an immune function comprised 12.0% (35 genes) of the dGATAe target list. This group includes several known immunity genes, including the gene that encodes the antifungal peptide Drosomycin, several highly transcribed but uncharacterized immune-induced molecules (IM1, -2, -3, and -10), the complement factor TepI, and Transferrin. Genes possibly involved in microbial recognition include PGRP-SC2, two leucine-rich repeat-containing proteins, and two proteins with predicted MD2-related lipid recognition domains. PGRP-SC2 is a peptidoglycan receptor shown to be gut-specific in a previous study (19). The genes CG5096 and CG18480 have leucine-rich repeat domains similar to those found in the extracellular domains of Toll and Toll-like receptors (20). CG12813 and CG3934 are homologous to the MD2 gene, which is thought to form a complex with LPS and Toll-like receptor 4 (TLR-4) in mammals (21, 22). A number of dGATAe targets also resemble cathepsins, cysteine proteases that process antigens for presentation to blood cells during an immune response (23).

Discussion

We have presented evidence that dGATAe is a mediator of immunity in the larval midgut. When misexpressed in the fat body, it causes a systemic immune response including constitutive expression of *Drs*, increased numbers of lamellocytes, melanized tissues, and scarring of the cuticle. We propose that GATA factors are crucial for determining tissue-specific immune responses in the fat body and gut.

Previous studies have established the importance of Serpent in mediating the systemic immune response in the larval fat body (9). Serpent is also essential for the differentiation of the fat body during embryogenesis; *srp* mutants are lethal and lack fat cell differentiation (24). Serpent is not merely a transient determinant of fat body development. We propose that dGATAe plays an analogous role in the anterior midgut: it functions as a tissue determinant in the embryo but mediates immunity in larvae.

dGATAe is expressed throughout the developing midgut during embryogenesis. As seen for *srp* in the fat body, *dGATAe* expression persists in the definitive midgut of feeding larvae. Thus, both *srp* and *dGATAe* might have dual roles in development and physiology. Early expression is required for tissue differentiation, and late expression is required for the immune response. *srp* mediates immunity in the fat body, whereas *dGATAe* mediates expression of specific immunity genes in the midgut.

Evidence that dGATAe functions in the early development of the midgut stems from microarray assays (Table 1). Misexpression of dGATAe in the fat body leads to ectopic induction of a number of genes required for digestion, including trypsin-like serine proteases, a sugar transporter, and genes involved in lipid metabolism. All of these genes display restricted expression in the midgut of developing embryos, in regions where dGATAe is also expressed. We propose that at least some of these genes are immediate and direct targets of dGATAe in the developing gut, and consequently, they are efficiently activated by dGATAe in the fat body.

dGATAe might also activate genes required for immunity gene expression in the anterior midgut of feeding larvae. By analogy to Serpent, dGATAe might activate different components of signaling pathways required for immunity. When larvae ingest pathogenic bacteria such as *E. carotovora*, these pathways are induced to trigger expression of *Drs*, *Dpt*, *Mtk*, and other immunity genes in the anterior midgut. When misexpressed in the fat body, dGATAe only moderately affects the levels of *Dpt* (array 1, 2.34-fold; array 2, 1.64-fold) or *Mtk* (array 1, 2.21-fold; array 2, 3.02-fold), although the regulatory regions of these genes showed GATA-dependent activity in the midgut. We attribute this low activity to the presence of repressors in the fat body that cannot be overcome by ectopic dGATAe.

It is possible that dGATAe-mediated immunity in the anterior midgut does not depend on the Toll signaling pathway, because none of the signaling components of this pathway are up-regulated in the fat body on misexpresion of dGATAe. This misexpression is nonetheless sufficient to induce *Drs* and *dro5* in

the absence of infection or injury (Fig. 4 and Table 1). We propose that dGATAe plays two roles in the differentiated midgut. One role is activating "housekeeping" genes that are required for digestion. The second role of dGATAe in the midgut is triggering unknown signaling pathways that lead to the activation of immunity genes such as *Drs*, *Dpt*, and *Mtk*. *Drs* may be especially sensitive to dGATAe in the fat body because it is "poised" for induction.

In summary, we have argued that dGATAe is critical for anterior midgut formation and function in a manner analogous to Serpent in the fat body. It is possible that the dGATAe immunity pathway is an evolutionarily ancient form of innate immunity. Under typical living conditions, the gut is the first line of defense, because ingestion is the most likely basis for contact with pathogens. The immunity signaling pathway(s) governing dGATAe activity is not yet known. However, ectopic expression of dGATAe in the fat body leads to the activation of a number of signaling components, including RhoL, Takl2, and Tetraspanins. The latter are integral membrane proteins that have been implicated in the immune responses of higher organisms, including antigen presentation (25). Future studies will assess the role, if any, of these genes in the gut-specific immune response.

Materials and Methods

Fly Strains. All flies were maintained at 25°C on standard cornmeal medium. Transgenic lines carrying *Dpt-lacZ* and *Mtk-lacZ* fusions and the method for creating point mutations in the reporter constructs are described in ref. 10. *UAS-dGATAe* was created by PCR amplification of a *dGATAe* cDNA (clone #LD08432; Open Biosystems, Huntsville, AL) with 5′ BglII-containing primer, (5′-GATC*AGATCT*ATGCCCATGCCCAGTCCCACTTTCCAGGCCCAAGCCCG-3′) and 3′ KpnI-containing primer (5′-GATC*GGTACC*TTAGTTATTCGATGATCGCTCTGGCAGACC-3′). The product was cloned as a BglII/KpnI fragment into the pP{UAST} vector (26). The *hsp70-GAL4* line was a gift from Fred Biemar (University of California, Berkeley, CA), and the *P{GawB}c754* line was from the Bloomington Stock Center (Department of Biology, Indiana University, Bloomington, IN).

***Erwinia* Infections and lacZ Staining.** 50 ml of *E. carotovora* was grown overnight, pelleted, and mashed into 4 g of standard fly food. Third-instar larvae were gently stirred in and left for one hour. Afterward, the entire mixture was transferred onto apple juice plates to sit overnight at room temperature. Larvae were then dissected, fixed, and stained as described in ref. 10. Three or more independent lines were compared for each construct.

RNA Isolation and RT-PCR. For RNA isolation dissected larval tissues were collected on ice. Samples were homogenized in a few drops of 1× PBS using a motorized pestle, then 1 ml of TRIzol (catalog no. 15596-026; Invitrogen, Carlsbad, CA) was added, and the samples were processed according to the manufacturer's instructions. The total RNA pellet was resuspended in RNase-free water. For RT-PCRs, the Access RT-PCR system (catalog no. A1250; Promega, Madison, WI) was used according to the manufacturer's instructions with the addition of 2 μCi [α-^{32}P]dCTP per reaction (1 Ci = 37 GBq). The final products were resolved on a 6% acrylamide (29:1 acyl/bis) gel with 0.5× TBE and quantified by using a Storm phosphoimager (Molecular Dynamics, Sunnyvale, CA). The primer pairs were as follows: *pnr*, 5′-ATGTACCACAGTAGCGCCGTTG-3′/5′-CGCCAAACTGGAAGTCCATGGCGCTCT-3′; *srp*, 5′-ATGCGGAACAACTTTGCGTTC-3′/5′-GCTGCTGCTGCTGATGGTGATGCAGTT-3′; *grn*, 5′-ATGGATATGACCTCAACAGCGG-3′/5′-GGGCATGCGGGATGTGTGCTGATGATA-3′; *dGATAd*, 5′-ATGAATAATGTACCACATAAGTTTCG-3′/5′-CGGGCACAGGCACGGAAACGGGTAT3′; *dGATAe*, 5′-ATGGTCTGCAAAACTATCTCACCG-3′/5′-TTCGCTGACGCCCGCTTGGCCCGTCT-3′; *dl*, 5′-ATGTTTCCGAACCAGAACAA-3′/5′-TCTTGCAGCCCTCCTTGCCAAC-3′; *Dif*, 5′-ATGTTTGAGGAGGCTTTCGG-3′/5′-GAACCGGCGGTGCGACCCTCGC-3′; *Rel*, 5′-ATGAATCAGTACTACGACCTG-3′/5′-ACGGTGGCACAGTGGCCGGAGC-3′; *spz*, 5′-CCAAGTATCGGCCACCACAATCCCCAGC-3′/5′-CCCTCAAGCCCTTTTTTGGGTACACCAG-3′; *Tl*, 5′-ATGAGTCGACTAAAGGCCGCTTCCGAG-3′/5′-AACCCTGTCGACCTCACCGATCCGCAAC-3′; *cact*, 5′-ATGCCGAGCCCAACAAAAGCAGCGG-3′/5′-GCTGATCCTTATCCTGTTCCTCGCTATC-3′; *Drs*, 5′-CCGTGAGAACCTTTTCCAATATGATGATGCAG-3′/5′-aTTGCAGCATAGAATATGTGTAAGTAGTGGAGAGC-3′; *rp49*, 5′-TCCGCCCAGCATACAGGCCCAAGATCGT-3′/5′-TTACTCGTTCTCTTGAGAACGCAGGCG-3′.

***In Situ* Hybridization.** Midgut tissue from third-instar larvae was fixed and hybridized with digoxigenin-UTP antisense RNA probes as described for *Drosophila* embryos in ref. 27. The probes correspond to nucleotides 2380–2640 for dGATAd, nucleotides 1930–2230 for dGATAe, nucleotides 242–602 for CG5096, nucleotides 329–689 for CG18480, nucleotides 235–595 for Tsp42Ep, and nucleotides 178–538 for Tsp29Fa.

Microarrays. For each array, ≈20 fat bodies were dissected, taking care to avoid the attached malpighian tubules and gonads. Total RNA was prepared by using the TRIzol method described above. The subsequent cDNA and cRNA synthesis reactions were carried out precisely according to Affymetrix instructions with their recommended reagents and kits. cRNA hybridization to *Drosophila* genome arrays were conducted by the Berkeley Functional Genomics Laboratory (University of California, Berkeley), and the data were analyzed with GeneTraffic software (Iobion Informatics, La Jolla, CA).

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